HISTOLOGIC COMPARISON OF REGENERATE BONE PRODUCED FROM DENTATE VERSUS EDENTULOUS TRANSPORT DISCS IN BONE TRANSPORT DISTRACTION OSTEOGENESIS

A Thesis

by

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ABSTRACT

Purpose: The purpose of this research was to quantify the number of blood vessels and nerves and mineral apposition rate (MAR) in native bone and compare it to the regenerate bone produced by bone transport distraction osteogenesis (BTDO).

Methods: Five adult foxhounds were subjected to the surgical removal of the anterior portion of the mandible. A bone transport reconstruction plate (BTRP-02, Craniotech ACR devices, LLC) device was used to transport the two segments created on each side of the mandible: One segment comprised an endodontically treated tooth, and the other was edentulous. After the bone transport distraction osteogenesis (BTDO) process was finished, 40-44 days of consolidation time was allowed before the dogs were sacrificed. The mandibles were resected and prepared for analysis. Histomorphometric and histologic analyses were performed for the regenerate and native bone.

Results: The histologic analysis showed no significant differences in the number of blood vessels (large or small) and nerves (large or small) between the native and regenerate bone. No significant differences were observed between the dentulous and edentulous regenerate bone. Confocal microscopy and Bioquant analysis showed significant differences ($P \leq 0.05$) in the MAR between the native bone and the regenerate bone, but no significant differences were observed between the dentate and edentulous regenerates.

Conclusion: The regenerate bone formed in the canine mandible by BTDO displayed a well-regenerated neurovascular complex within the alveolar canal, containing large and small blood vessels and nerves that were compared to those present in the native bone. Mineralization of the regenerate bone occurred at a rate higher than that of native bone.

Key words: Bone Transport, Distraction Osteogenesis, Blood Vessels, Nerves, Mineral Apposition Rate

DEDICATION

To my mom and dad for all the love, sacrifice and support throughout all this process. To my brothers for always being there and to all my family and loved ones for the unconditional support. To God for never letting me give up.

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CHAPTER I

INTRODUCTION

Background and Significance

Defects in the maxillofacial region can be caused by diverse conditions and situations. These defects may include congenital, pathologic or iatrogenic conditions such as orofacial clefts, tumor excision and post radiation necrosis(Kalantar-Hormozi and Khorvash 2006; Rashid, Zia-ul-Islam et al. 2006). Maxillofacial defects including bone loss can also result from blast injuries, high-impact trauma, excision of some benign tumors or repeated surgical debridement for the treatment of chronic osteomyelitis or osteoradionecrosis (Mehta and Deschler 2004; Elsalanty, Taher et al. 2007). Various factors could be the cause of a mandibular defect but the most common indication for mandibular reconstruction still remains ablative surgery for the neoplastic processes of the oral cavity(Cordeiro, Disa et al. 1999; Foster, Anthony et al. 1999; Rana, Warraich et al. 2011). The tumors affecting the mandible may be squamous cell carcinoma, ameloblastoma, pindborg tumor, adenomatoid odontogenic tumor, central giant cell granuloma and odontogenic myoxoma, among others(Rana, Warraich et al. 2011). According to Rana et al., oncological resection accounted for 42.1% of the incidence of mandibular bony defects, followed by post traumatic defects (24.7%), post-operative defects after gap arthroplasty in the TMJ ankylosis (19.6%), and osteomyelitis of the mandible (13.5%) (Rana, Warraich et al. 2011).

Reconstruction of mandibular defects is one of the most challenging surgeries because of the many problems the injury causes. The main problems are a limited range of motion for lateral and protrusive movements, malocclusion, proprioception, mastication and deglution(Curtis, Taylor et al. 1975; Cordeiro, Disa et al. 1999; Foster, Anthony et al. 1999). In addition to these conditions, the treatment of cancer patients involve not only the surgical procedure of removing hard and soft tissue from the face and oral cavity, but it may be combined with radiotherapy, which is believed to compromise bone and soft tissue regeneration(Marx and Johnson 1987; Dudziak, Saadeh et al. 2000; Nussenbaum, Rutherford et al. 2003).

Patients whose mandibles are removed undergo the following physiological changes: deviation of the mandible to the resected side because of unimpeded muscle pull, limited range of motion in lateral and protrusive movements, some return to midline position on opening and closing due to the actions of the remaining contralateral muscles of mastication, and impairment of occlusion and proprioception (Curtis, Taylor et al. 1975).

In general, the functional complications after resection will be affected by many different factors. In our study, we proposed a surgery that simulates the majority of these factors by removing the entire anterior segment of the mandible of the dogs. The aesthetic alterations and functional losses that can occur with mandibular defects will depend greatly on the size and the location of the defect. Posterior mandibular defects are generally better tolerated. When the defect involves the symphysis or the anterior body of the mandible, a more considerable deformity and compromised function occur

(Urken, Weinberg et al. 1991). These defects are the hardest to reconstruct because of the difficulty to restore the curve of the mandible. In this study, we proposed a complete anterior resection to test a new bone transport reconstruction plate (BTRP-02) and then reconstruct the mandible. The BTRP uses two transport segments, one from either side of the mandible to be transported anteriorly to the midline. Anterior mandibular reconstruction should achieve certain goals to be considered successful. Several authors have suggested different parameters regarding achieving a successful surgical procedure. Rana et al. suggested that the functions of chewing, swallowing, speech articulation and oral competence should be achieved. Researchers have also suggested that the main goal of mandibular reconstruction is to restore the patient to the previous state of function. The surgeon must restore bony continuity and facial contour, maintain tongue mobility and attempt to restore sensation to the denervated areas (Rana, Warraich et al. 2011). Other authors advocate that accurate classification of the defect, immediate and complete wound closure, establishment of mandibular continuity, establishment of an osseous alveolar base for a prostheses and a good aesthetic outcome depends on accurately reproducing the shape of the mandible. Correcting the alveolar and soft tissue deficiencies in preparation for prosthetic reconstruction and support for endosseous implants should also be considered when a mandibular reconstruction is attempted (Vikram, Strong et al. 1984; Urken, Weinberg et al. 1991; August, Tompach et al. 2000; Kroll and Reece 2001; Bak, Jacobson et al. 2010).

Today mandibular reconstruction is the result of almost 60 years of refinement of the different techniques. New microsurgical techniques, biomedical advances in plating technology and instrumentation, as well as an improved understanding of the donor site, have made mandibular reconstruction using flaps or grafts reliable(Mehta and Deschler 2004). Mandibular defects may be fixed with alloplastic material, non-vascular autologous tissues, vascular autologous tissues or a combination(Koch, Yoo et al. 1994; Schrag, Chang et al. 2006).

Current mandibular reconstruction implies the use of bone containing free flaps. However for patients with advanced oral cancer and a poor prognosis, time is very important; in these cases, a combination of mandibular reconstruction plates with soft tissue free flaps is an alternative(Urken, Weinberg et al. 1994; Blackwell, Buchbinder et al. 1996; Blackwell, Buchbinder et al. 1997).

Alloplastic material, which primarily consists of steel or titanium plates, was used because the reconstruction was simple, fast and required no donor site. The most commonly used alloplastic implants for mandibular reconstruction are bone plates and screws. The use of mandibular plates is indicated in patients with poor performance or in cases where the soft tissue defect of the oral cavity is more extensive than the bony mandibular defect(Genden and Haughey 1996). The only concern is that alloplastic materials have unacceptably high rates of fracture, exposure, and infection. There is a higher incidence of plate failure in defects located anteriorly and in patients receiving radiation (Kim and Donoff 1992; Spencer, Sizeland et al. 1999; Wei, Celik et al. 2003).

Due to the complications seen with reconstruction plates and alloplastic grafts and materials, autografts have become the gold standard for mandibular reconstruction. The success of primary reconstruction is improved dramatically if circulation is restored immediately after the bone graft (Urken, Buchbinder et al. 1998). These grafts, known as “free tissue transfers,” including vascularized free tissue transfer, vascularized osseous free tissue transfer, vascularized free bone flaps or vascular autologous tissue transfer, may also include soft tissue for reconstruction of accompanying mucosal or cutaneous defects (Nussenbaum, Rutherford et al. 2003; Mehta and Deschler 2004; Schrag, Chang et al. 2006). Vascularized free bone flaps are the gold standard for being the most reliable and predictable modality to restore the form and function of the lost mandibular segments. The most common donor sites for osseous free-tissue transfer include the fibula, scapula, iliac crest, and radius (Cordeiro, Disa et al. 1999; Foster, Anthony et al. 1999). This procedure is indicated for segmental bone defects, composite bone grafting and situations involving previous or planned radiation therapy (Genden and Haughey 1996; Urken, Buchbinder et al. 1998; Schrag, Chang et al. 2006).

Multiple advantages make vascularized free bone flaps the preferred reconstructive modality today. The advantages include: 1) a rapid predictable bony union by appositional healing instead of the process of creeping substitution that occurs in bone grafts; 2) minimal bony resorption and the actual formation of new bone; 3) resistance of the flaps to infection and the detrimental effects of radiation; and 4) excellent long-term aesthetic and functional outcomes (Berggren, Weiland et al. 1982; Klein, Stevenson et al. 1991; Fujimaki and Suda 1994; Mehta and Deschler 2004; Rana, Warraich et al. 2011).

Non-vascularized autogenous bone grafts are widely used for mandible reconstructions and are relatively easy to perform due to the large quantities of bone that are available from several donor sites (Taylor 1982; Wersall, Bergstedt et al. 1984; Foster, Anthony et al. 1999). The sources for these types of grafts can be divided into local and distant sites. Normally, if the defect is small, local or intra-oral donor sites are often sufficient for the surgery. On the other hand, when a moderate to substantial amount of bone is required, distant or extra-oral sites are usually preferred. It has also been reported that greater failures have been observed in defects larger than 9 cm (Pogrel, Podlesh et al. 1997). Historically, autogenous bone grafts come from the calvaria, rib, ilium, tibia, fibula, scapula and radius (Foster, Anthony et al. 1999; Mehta and Deschler 2004) Mehta and Deschler 2004). All of these sources produce highly successful grafts if circulation is restored immediately during the reconstruction.

For this reason, vascularized bone grafts (VBG) have become the mainstay for maxillary reconstruction through the years. In spite of the great success of this procedure, failures can still be observed (August, Tompach et al. 2000). This highly demanding technique requires specialized surgical teams because of the complexity of the primary surgery. The surgeries are therefore lengthy and expensive. Another disadvantage is the donor site morbidity (Holzle, Kesting et al. 2007). Pain, instability, motor weakness and nerve injury have all been reported as morbidity issues. Vail et al. reported objective motor weakness, subjective discomfort in the ankle and other sites in the leg, and sensory abnormalities in the lower limb from which the graft had been obtained. In this study, the researchers also mentioned problems regarding restriction of motion of the fibula, pain related to the incision and a delayed healing related to the diminished vascularity after the surgery at the donor site (Vail and Urbaniak 1996). For this reason, new treatment options are constantly being explored including bone transport distraction osteogenesis (BTDO), which has proven its effectiveness in reconstructing bone defects (DeCoster, Gehlert et al. 2004).

However, many physicians still consider BTDO to be a suboptimal procedure because many of the devices have high failure rates and non-esthetic outcomes. Mandibular bone transport (MBT) appliances are designed with four different parts: the frame, the bone transport unit, the distraction activating mechanism and the activation arm. These parts provide mechanical stiffness, strength, and support along with the protection of the new bone tissue and support of the transport disc of bone (Zapata, Elsalanty et al. 2010). The distraction devices can be divided into internal and external types (Imola and Tatum 2002). The internal appliances have the distraction mechanism implanted below the skin or mucosa and are fixed to the bone using conventional metal plate and screw hardware. A connecting rod passes from the distraction mechanism and exits through the skin or mucosa to allow activation. The intraoral devices have improved stability, improved patient compliance, lower infection rates, leave no scars and may contribute to soft tissue expansion (Chin and Toth 1996; Rubio-Bueno, Padron et al. 2000; Primrose, Broadfoot et al. 2005). The external devices, on the other hand, are positioned outside the skin or mucosa and are secured to bony segments using transcutaneous or dental fixation. These extraoral devices are preferred when complicated three-dimensional bone reconstructions are required (Labbe, Nicolas et al. 2005; Ortakoglu, Karacay et al. 2007). The novel device proposed in this study is an internal device used to reconstruct a three-dimensional bone defect (Figure 1).

One of the biggest challenges in reconstructing the mandible is encountered with the curve of the mandibular symphysis. According to Sacco et al., up to 2007, no internal curved distractors in these positions had been assessed in animal models or had therefore been made available for human use (Sacco and Chepeha 2007). In 1994 Annino et al. were the first to describe the reconstruction of a mandibular symphyseal defect (Annino, Goguen et al. 1994). They used two transport discs, one from each of the mandibular bodies, which met at the midline. They concluded that by means of an arced trifocal device, distraction osteogenesis is a viable option to reconstruct the mandible after anterior mandibulectomy. They stated that current symphyseal reconstruction is primarily limited by the lack of an appropriate distraction apparatus. Their device was less than ideal; it had extraoral projections around the mandible, and linear scars were left as the pins were distracted forward. Therefore, a better distraction apparatus, such as an intraoral device was warranted to 1) facilitate patient self-management; 2) minimize or not create unsightly soft tissue scars; and 3) assist in the restoration of dentition and occlusion (Annino, Goguen et al. 1994).

In 2004, Herford used an internal device, the "plate-guided distraction device," in which a reconstruction plate is placed to bridge the mandibular continuity defect. All patients achieved both hard and soft tissue formation with this device. Herford emphasized that distraction osteogenesis around a curve presents certain challenges. He observed that the plate will follow the curve but as the distractor moves along the curve, the regenerated tissue will straighten (Herford 2004). In 2006, Hibi et al. used an internal device in which they overcame the limitations regarding length and position of the defect that the researchers had with the device (Hibi and Ueda 2006). In 2008, Zhou et al. described an internal transport distraction device, which comprised an internal square-bodied bow, a transport plate, a traction mechanism, and two stabilizers. The authors concluded that a curved mandibular angle defect could be reconstructed by transport distraction along an internal bow, using a cable to apply the traction force (Zhou, Shang et al. 2008).

A new device, the mandibular bone transport reconstruction plate (BTRP) for distraction osteogenesis, was introduced by Elsalanty and colleagues. The BTRP is an intraoral device that depends on a modified reconstruction plate as a transport track, along which the transport bone disc travels towards the docking site (Elsalanty, Zakhary et al. 2009). This newer version of the BTRP allows two forms of distraction after complete resection of the anterior mandible. Transport can occur from one side of the mandible, around the front of the symphysis to a docking site on the other side of the mandible. Alternatively, two transport units can be attached to the same reconstruction plate on either side of the mandible, and transport segments can be moved until they meet at the symphysis, and perhaps fuse, creating a new symphysis.

The biology of bone transport osteogenesis is similar to that of distraction osteogenesis (DO). DO is considered to be one of the greatest developments in craniofacial surgery in the past decade. It is a method of tissue regeneration in which new bone is formed within the gap between two opposing bone segments that are gradually separated by externally applied forces (Ilizarov 1971; Ilizarov 1989; Ilizarov 1989). The technique was described initially by Codivilla, (Codivilla 2008) and was popularized by Ilizarov (Ilizarov 1971), who examined the biological principles of distraction osteogenesis and stated that the new bone formed in response to the tension-stress effect induced by mechanical strain (Ilizarov 1989; Ilizarov 1989). He also established that the best quality of new bone was achieved when the bone marrow and periosteum were preserved. In addition, he stated that the degree of stability of fixation affects bone formation: the better the stability, the less cartilage or fibrous tissue formed in the regenerate bone (Ilizarov 1989).

The application of DO in the maxillofacial complex began in 1973 with Snyder's study on maxillary elongation in dogs (Snyder, Levine et al. 1973; Pereira, Luiz de Freitas et al. 2007). In humans, distraction osteogenesis has been used for several procedures. These include palatal expansion, mandibular symphysis elongation, correction of congenital facial abnormalities such as hemifacial microsomia, treatment for cleft patients, repair of continuity defects of the mandible, alveolar crest augmentation, and mandible reconstruction after tumor resection (Bell and Epker 1976; Bell, Harper et al. 1997; Chiapasco, Brusati et al. 2000; Figueroa, Polley et al. 2004; Fukuda, Iino et al. 2004). Many different sizes and locations of the defects can be treated, depending on the severity of the initial lesion.

Different techniques are being used currently to reconstruct these defects, based on the location and size of the segment to be restored. DO has been classified as monofocal, bifocal, and trifocal, according to the number of foci at which osteogenesis occurs (Cohen 1999). Friedman and Costantino (Costantino Pd 1993) found that, in the monofocal classification, a surgical fracture creates a gap between two bone surfaces where the healing events will happen for posterior traction of the separated bone segments. This approach is mainly used for vertical alveolar augmentation prior to implant placement. In the bifocal approach (BTDO), a surgically produced bone segment is transported along the defect to solve a continuity problem; the moving segment is called the "transport disc." The current approach is utilized for mandibular reconstructions after tumor ablation. Finally, in the trifocal classification, two transport discs are created, one on each side of the defect. The discs are then moved until they meet. This method is usually used for major mandibular defects (Costantino Pd and et al. 1990; Costantino Pd 1993). The current study reports on the use of trifocal DO, in which a transport disc is created on each side of the mandible, and the two segments are transported to the midline.

In BTDO, a segment of bone is osteotomized adjacent to the defect and is moved across the defect with a mechanical device. After this initial osteotomy and the distractor device fixation procedures, the DO consists of three phases or steps. The first one is the latency phase, which is the period between the bone division and the device's activation. This phase occurs during the time allowed for the initiation of callus formation after the osteotomy. This latency period can last from 0 to 5 days or 7 to 14 days (Imola and Tatum 2002; Pereira, Luiz de Freitas et al. 2007; Sacco and Chepeha 2007) and should be planned in a way in which calcification is not permitted and the formation of a primary osseous callus is avoided (Ilizarov 1989; Ilizarov 1989; Pereira, Luiz de Freitas et al. 2007). The second step is the distraction or transport phase in which the bone edges are distracted once or twice a day with the aid of the distraction device. During this phase, the stretching stimulates tissue neo-formation at the distraction gap in a direction parallel to the vector force. The frequency of activation and distraction rate has been agreed by the majority of authors to be 1 mm/day (Ilizarov 1989; Ilizarov 1989; Ilizarov 1990; Bell, Harper et al. 1997; Ilizarov 1997; Chiapasco, Brusati et al. 2000; Sacco and Chepeha 2007). The third step is the consolidation phase, occurring after the end of the distraction when the fragments are stabilized at an ideal position. The distractor remains in place in this phase but is inactive and is used as a rigid fixation device. The consolidation time varies from 4 to 12 weeks but 8 weeks seem to be long enough to allow for the new bone formation (Chiapasco, Brusati et al. 2000; Rubio-Bueno, Padron et al. 2000; Imola and Tatum 2002; Sacco and Chepeha 2007).

Not many studies examining the new bone and surrounding soft tissues created as regenerate bone are found in the literature. From the few studies published, Panikarovskii et al. performed the first significant histologic evaluation of mandibular distraction regenerates. These researchers described a fibrous interzone in the central region of the distraction gap with collagenous fibers and capillaries oriented parallel to the direction of the distraction. In the new bone, the trabeculae were longitudinally oriented and grew towards the fibrous interzone. They found a relationship between the vector of distraction and the orientation of the primary osteons (Panikarovskii, Grigor'ian et al. 1982). Karp conducted a study on a comprehensive analysis of the histology of the distraction regenerate at different stages of formation. In this study, they described the distraction gap in terms of four zones: 1) a central zone of fibrous tissue that consists of longitudinally oriented, parallel strands of collagen; 2) a zone of extended bone formation that is characterized by fibroblasts and undifferentiated mesenchymal precursor cells in direct continuity with osteoblasts on the surface of early bone; 3) a zone of bone remodeling consisting of portions of bone resorption and apposition; and 4) a zone of mature bone made up of early cortical bone located adjacent to the mature bone in the unexpanded areas of the mandible (Karp, McCarthy et al. 1992). Zapata et al. indicated that the new regenerate bone formed in their study had the basic haversian systems parallel to the vector of distraction in the regenerate cortical bone and the same pattern is present in the control cortical bone with the difference being that their orientation is parallel to the base of the mandible. They concluded that there is no significant difference between the new regenerate and control cortical bone, except for the level of mineralization reached during this specific consolidation period (Zapata, Halvachs et al. 2011). Using micro computed tomography (μ-CT), Kontogiorgos et al. found that regenerated mandibular bone continued to mineralize from 12 to 18 weeks after consolidation, but after this step it did not reach the levels of control bone. Regenerated bone has thicker, denser and more numerous trabeculae than native bone. The architecture of the regenerated bone was mainly trabecular with an outer thin layer of cortical bone, whereas the control bone demonstrated well defined cortical and trabecular bone regions (Kontogiorgos, Elsalanty et al. 2011).

Distraction of dentate bone transport segments would allow the preservation of intact tooth structure in surgeries where massive tissue loss is problematic. Dentoalveolar distraction has been used to rapidly move canines through extraction sites in humans (Moore, Campbell et al. 2011). The technique performed involves the extraction of the maxillary first premolar, then corticotomies around the root of the canine, and removal of the buccal plate and the interseptal bone distal to the canine. This segment is then mobilized and rapidly distracted into the new extraction site. The purpose of the osteotomies is to remove the mesial and the apical bone because it is thought that this procedure will promote angiogenesis and bone healing from an increased vascular supply to the dentoalveolar segment. This vascular increase added to the surrounding bone is what differentiates dentoalveolar distraction from traditional distraction osteogenesis (Moore, Campbell et al. 2011). In one study that examined the regenerate bone when a tooth is present in the distraction transport segment, it was observed that the regenerate bone produced more healing on the lingual than on the buccal side (Spencer, Campbell et al. 2011). The lingual bone included intact cortical bone with variable amounts of trabecular bone. However, the biologic properties of regenerate bone after dentoalveolar distraction have not been well described in the literature. For this reason, it is proposed that regenerate bone, where a tooth was present in one of the transport discs be compared to regenerate bone formed by an edentulous transport disc.

Mineral apposition rate (MAR) is a measurement performed by quantifying the distance between two fluorochromes injected into the dogs at specific times. Fluorescence is defined as the property possessed by certain substances to convert short wavelengths of light into radiation of longer visible wavelengths, which is a process of absorbing radiant energy and reradiating a portion of this energy in wavelengths different from those absorbed. Primary fluorescence (autofluoresence) is the inherent capacity of substances to fluoresce when exposed to an exciting ultraviolet light source. Secondary fluorescence is the fluorescence induced in substances by the application of fluorescent compounds or dyes (fluorochromes) (Boyne and Kruger 1962). Tetracyclines are autofluorescent antibiotics that bind to immature bone mineral at the osteoid seam/mineralized tissue interface. In 1957, it was demonstrated that following parenteral administration of tetracycline antibiotics, fluorescence was observed in regions of new bone proliferation (Milch, Rall et al. 1958; Tobie 1958). It was of considerable interest that the fluorescence was typically present in regions characterized by newly proliferated bone tissue, in contrast to already formed bone, which displayed only autofluorescence. Periosteal surfaces showed intense fluorescence, which sharply contrasted with regions of earlier bone deposition. In a similar way, newly formed Haversian canals were distinguished from those formed earlier (Milch, Rall et al. 1958). The administration of two time-spaced courses of tetracycline results in the appearance of two parallel fluorescent bands or labels at sites of bone mineralization. The use of tetracycline permits the quantification of the cellular rate of mineralization, which is the rate of matrix calcification occurring at any average point of bone formation and represents the mean distance between the two parallel fluorescent labels divided by the time between doses. It also allows the determination of the total rate of bone calcification by using the linear extent of mineralization. This variable reflects the extent of bone surface involved in the mineralization process and represents the percentage of bone surface containing a fluorescent label (Fallon and Teitelbaum 1982).

Cope measured the total surface area in the first report to establish a mineral apposition rate for craniofacial distraction osteogenesis (Cope and Samchukov 2000). The interlabel distance, which gives the mineral apposition rate when divided by the number of days between injections, was measured from the midpoint of the first label to the midpoint of the second label. At least 20 distances were measured, and all lines were drawn perpendicular to the tangent of the label. The results in this study indicated that bone formation gradually increased from the end of distraction to the fourth week of consolidation, and then it remained constant until sometime before the eighth week. By four weeks, the mineral apposition rate was 2.67 microns per day (Cope and Samchukov 2000). These studies demonstrated that MAR could potentially be used to compare bone regenerate formed in response to different treatment regimens. In another study conducted by Williams et al., researchers used fluorochromes injected at different time periods to investigate bone formation in distraction sites at various times following slow, moderate, and rapid rates of mandibular distraction in adult rats. They found no significant difference in MAR for the different distraction rates (Williams, King et al. 2005). In our study, we intended to transport bone segments from either side of the mandible toward the midline. One of the transport segments contained an endodontically treated tooth, and the other contained an extraction socket. The hypothesis tested was that a dentate transport segment will remain more intact than an edentulous segment, and will thus produce more rapid bone and soft tissue regeneration. MAR was used to measure whether more rapid mineral apposition occurs in dentate versus edentulous BTDO. No previous studies were found testing the effect of dentate versus edentulous transport discs on MAR in regenerate bone.

In mandibular BTDO, the blood supply and nerves within the alveolar canal ahead of the transport unit are disrupted, either by injury or surgical resection. No studies on mandibular BTDO have reported whether the blood vessels and nerves remain intact within the alveolar canal of the newly formed regenerate, or whether new structures appear after bone transport is completed. Few studies were found examining the histology of nerves and blood vessels in new regenerate bone after DO. An increased blood supply may be important in the formation of regenerate bone, healing at the docking site, and healing of other lesions within the affected zone. This increased blood supply is mediated by the formation of new blood vessels. In their study on DO, Carvalho et al. suggested that multiple families of genes contribute to angiogenesis within the regenerate. Angiogenesis is the physiological process involving the growth of new blood vessels from preexisting vessels (Carvalho, Einhorn et al. 2004). DO is a highly reliable method for the regeneration of bone deficiencies, which depends upon the local blood supply, stable fixation , and gradual stretching. However, DO can fail in at least four ways. Two of these ways are related to a deficient blood supply: 1) Ischemic fibrogenesis that occurs when there is inadequate blood supply during the distraction process, and 2) Cystic degeneration, which happens when there is blockage of venous outflow from the system (Aronson 1994). It has been demonstrated that the overall blood supply to the distraction zone as measured by quantitative technetium scintigraphy, was seven times greater than that occurring in the normal contralateral side during distraction. It subsequently remained at approximately three times the normal level for the next three months during remodeling. It was also observed in this study that the overall blood flow to the distal half of the regenerate remains less than that in the proximal half on both the distracted and nondistracted sides (Aronson 1994). Pacicca et al. suggested that during DO, angiogenesis occurs first, followed by organized cell growth oriented to the new vessels. Once an appropriate blood supply is established, the system stops endothelial differentiation and switches to an osteogenic process (Pacicca, Patel et al. 2003). In the current study, the number of blood vessels present was counted at three selected sites along the regenerate bone. The blood vessels were counted in the alveolar canal within the native bone closest to the cut edge, creating the transport segment, in the regenerate closest to the native bone, and in the regenerate just behind the transport segment. To our knowledge, the only study similar to this one was done by Rowe and colleagues. In their experiment counting blood vessels, they found an intense angiogenic response associated with membranous bone that occurs during the early stages of distraction. The newly formed blood vessels were associated primarily with the peripheral areas of the osteotomized bone edges. In this rat model, the number of blood vessels noted in the mandible at this time point was significantly greater than that noted at the end of distraction or in consolidation. They noted the highest concentration of newly formed blood vessels in areas of bone adjacent to the periosteum (Rowe, Mehrara et al. 1999).

Restoration of sensation within the regenerate tissues is important to regaining normal function and esthetics. It has been reported that acute and chronic alterations to the nerves are caused during DO. In his study, Haftek concluded that "nerve trunks possess a high degree of elasticity, which is mainly a feature of the epineurium. Initial elongation of the nerve is due to extension of the epineurium and straightening of the funiculi and of the nerve fibres. Such elongation is physiological in the sense that it does not affect the nerve fibers" (Haftek 1970). He also concluded "that the first structure to rupture is the epineurium and this occurs when the nerve trunk has reached its limit of elasticity. Before this rupture of the epineurium occurs, the damage to the nerve fibers is either neurapraxia or axonotmesis, because the endoneurial sheaths and schwann tubes remain intact" (Haftek 1970). Block et al. (1993) reported in their study with mongrel dogs that in nerve compression model, most demyeliation occurs at the interface between the compressed and noncompressed segments of the nerve. Therefore, myelination may be minimal in segments of gently stretched nerves subjected to equal pressures.(Block, Daire et al. 1993)

Slow nerve stretching, as in the Ilizarov method of DO, may result in minimal injury because less axonal tearing and less complete compression of the vasa nervorum takes place. Researchers found that the nerve injury due to slow traction associated with DO is mild (Block, Daire et al. 1993). Karp found that myelinated fibers were absent in specimens of the inferior alveolar nerve taken from the operated side of the mandible, whereas the nerve on the unoperated side showed normal structure and myelination. An experiment conducted by Karp and colleagues, during surgery, attempted to conserve the inferior alveolar nerve and as much periosteum as possible (Karp, Thorne et al. 1990). These findings suggest that there was nerve injury during DO. Makarov et al. performed electrophysiologic studies in dogs to evaluate the inferior alveolar nerve function during DO of the mandible. They also showed that inferior alveolar nerve (IAN) injury is associated with either the osteotomy or segment fixation by the screws. This conclusion was evident because they observed that neural structures were frequently found to be pierced or displaced by the screws. The results of the study suggest that distraction osteogenesis may be associated with significant electrophysiologic abnormalities of the inferior alveolar nerve function (Makarov, Harper et al. 1998). In another study by Makarov et al., it was found that the most significant cause of peripheral nerve dysfunction was encroachment on the nerve by transfixing wires. An extensive soft tissue fibrosis with subsequent nerve involvement was also found to be a secondary cause (Makarov, Birch et al. 1996).

Ilizarov has stated that it is important to try to conserve the blood vessels, periosteum, and nerves as intact as possible during the surgeries associated with DO (Ilizarov 1989). To assess the effects of severing the nerves and blood vessels anterior to the transport disc within the alveolar canals prior to BTDO, we performed a nerve count within the regenerate and native bone. It was also important to observe if there was any difference in this study between the numbers of nerves and blood vessels present in regenerate formed from dentate versus edentulous segments.

After the two transport discs come together at the symphysis, fusion of the soft tissues and bone should occur. However, non-unions at these docking sites are an ongoing problem. Nagashima et al. reported that half of the foxhounds used in their study did not have union at the docking site. It was observed that the transport discs had irregular bone spikes anteriorly, which prevented the advancement of the transport segment all the way to the docking site. Also, there was interposition of soft tissues between the advancing edges of the transport disc and the recipient bone segment (Nagashima, Rondon-Newby et al. 2012). Histologic examination of the midline tissues was performed in the present study to establish whether or not bony union has occurred.

Main Goal

The main goal of this study was to analyze the mineral apposition rate in regenerate bone and the number of blood vessels and nerves in the regenerate alveolar canal in the newly formed bone created by BTDO with two transport discs, one containing an endodontically treated tooth and the other made edentulous by extracting the tooth.

Hypothesis

Native bone and regenerate bone behind dentate transport segments will have significantly greater MAR and more blood vessels and nerves after the distraction osteogenesis consolidation phase, compared to native and regenerate bone behind the dentate and edentulous transport segments.

Specific Aims

- Compare bone mineralization apposition rates (MAR) between the native bone and the regenerate bone on each side of the mandible to establish the baseline MAR levels of the regenerate relative to native bone after 6 weeks of consolidation.
- Compare the MAR of the bone on the edentulous versus dentate transport segment sides to establish whether a transport segment with an endodontically-treated tooth produces a greater MAR than an edentulous transport segment.
- Compare the number of blood vessels in the regenerate and native bone behind the dentate and edentulous transport segments to test whether the dentate transport segments foster more blood vessel formation than the edentulous transport segments.
- Compare the number of nerves in the regenerate and native bone behind the dentate and edentulous transport segments to test whether the dentate transport segments foster more nerve formation than edentulous transport segments.

CHAPTER II

HISTOLOGIC COMPARISON OF REGENERATE BONE PRODUCED FROM DENTATE VERSUS EDENTULOUS TRANSPORT DISCS IN BONE TRANSPORT DISTRACTION OSTEOGENESIS

Introduction

Defects in the maxillofacial region can be caused by diverse conditions that may include congenital, pathological or iatrogenic conditions such as orofacial clefts, tumor excision and post radiation necrosis. Different techniques are currently being used to reconstruct these defects, depending on the location and size of the segment to be restored. One technique is bone transport distraction osteogenesis (BTDO), in which a segment of bone is osteotomized adjacent to the defect and is moved across the defect by the use of a mechanical device.

In BTDO and distraction osteogenesis in general, great importance has been given to the investigation of how new bone forms in response to BTDO. Previous research studies have placed special emphasis on characteristics related to the quality and quantity of the new regenerate bone formed (Zapata, Halvachs et al. 2011; Zapata, Opperman et al. 2011; Nagashima, Rondon-Newby et al. 2012). Nagashima et al. concluded that after four weeks of consolidation, the histologic and biomechanical characteristics of the new bone showed that it was less mature than the control bone (Nagashima, Rondon-Newby et al. 2012). In a study conducted by Zapata et al, the researchers created a unilateral defect, utilized BTDO to create new regenerate bone, and used micro-CT and histologic analyses to examine the bone after twelve weeks of consolidation. It is important to note that one of the main findings of this study included the observation that the biomechanical and histological characteristics of the regenerate bone were similar to those of the control bone. The differences found could be attributed to the lack of complete mineralization of this new regenerate bone (Zapata, Halvachs et al. 2011; Zapata, Opperman et al. 2011). Moreover, a three-dimensional evaluation of the regenerated bone created by BTDO showed that the bone continues its maturation process through the consolidation period and that the main difference between the native bone and regenerate bone is the thicker outer layer of cortical bone observed in the more mature native bone (Kontogiorgos, Elsalanty et al. 2011).

Although many studies have been conducted about the way the new regenerate bone matures, to our knowledge there is limited evidence in the scientific literature that explores soft tissues, including blood vessels and nerves, created in the new regenerate bone using BTDO. In addition, regeneration of the alveolar canal, including the inferior alveolar nerve and associated blood vessels during BTDO in the mandible, has not been reported.

In mandibular BTDO, the blood supply and nerves within the alveolar canal ahead of the transport unit are disrupted, either by injury or surgical resection. It is known that in regular distraction osteogenesis where the blood supply is not completely eliminated on one of the sides of the defect, the inferior alveolar artery and nerve have successfully regenerated (Costantino Pd and et al. 1990; Karp, Thorne et al. 1990; Block, Daire et al. 1993). Particularly, in their study creating new bone in mongrel dogs with distraction osteogenesis methods, Block et al. observed that the inferior alveolar nerve spanned the regenerate bone without any continuity defects (Block, Daire et al. 1993). In the same way, Karp et al. performed an osteotomy aiming to preserve the neurovascular bundle during DO and observed the regeneration of the alveolar nerve, although no myelinated fibers were observed in this neurovascular bundle (Karp, Thorne et al. 1990). On the other hand, Costantino et al. intentionally cut the neurovascular bundle and found that, regardless of this resection, the alveolar artery was regenerated in the new bone, but not the inferior alveolar nerve (Costantino Pd and et al. 1990). These overall findings from previous studies, suggest that injury and repair of the blood vessels and nerves can occur and could be potentially affected by the osteotomy technique and how carefully the neurovascular bundle is conserved during the surgery (Makarov, Birch et al. 1996; Makarov, Harper et al. 1998). However, there were no reports found that evaluated how complete resection of the mandible, in which the blood supply anterior to the distraction segment is eliminated, affects the formation of this neurovascular bundle.

An increased blood supply may be important in the formation of regenerate bone. This increased blood supply will be mediated by the formation of new blood vessels. It has been shown in DO that the overall blood supply to the distraction zone was seven times greater than that occurring in the normal contralateral side during distraction and then remained approximately three times greater than normal during the consolidation phase (Aronson 1994). To our knowledge, the only study that quantified the number of blood vessels in the regenerate was done with DO by Rowe and colleagues. In their experiment on blood vessel count, they demonstrated an intense angiogenic response associated with membranous bone that occurred during the early stages of distraction. In this rat model, the number of blood vessels noted in the mandible at this time point was significantly greater than that noted at the end of the distraction or in consolidation. They observed the highest concentration of newly formed blood vessels in areas of bone adjacent to the periosteum (Rowe, Mehrara et al. 1999). In BTDO, no reports were found that quantified the number of blood vessels in the regenerate bone, and none reported if the presence of a tooth was going to affect the formation of blood vessels.

Another concern with patients going through mandibular resection is the prosthetic rehabilitation. One of the primary goals of the treatment is restoration with an acceptable occlusion. The degree of success is related to the location and extent of the defect as well as the presence or absence of natural teeth (Desjardins 1979). One of the most common postoperative rehabilitative needs is tooth placement, which is often limited by the lack of a weight-bearing surface for the prosthesis. A general principle that should be followed is to try to do everything possible to increase the potential postoperative health status and potential usefulness of the remaining teeth. Teeth that would normally be considered nonrestorable may become extremely valuable and critical for the stability and retention of the prosthesis (Taylor, clinical maxillofacial prosthetics chapter 7). There is limited evidence showing that a tooth in the transport disc will become critical for the prosthetic rehabilitation and will have any influence on the neurovascular bundle and bone in the new regenerated segment. However, as a first step, it is important to assess the quality of the regenerate produced by a dentate transport segment, and whether the presence of a tooth affects nerve and blood vessel regeneration.

The purpose of this research study was to analyze the histology of newly formed mandibular bone created by BTDO with two transport discs: one with an endodontically treated tooth and the other being edentulous in a complete anterior resected mandible. The specific aims of the study were to compare: 1) Bone mineral apposition rates (MAR) between native bone and regenerate bone on each side of the mandible; 2) the MAR of the regenerate bone on the edentulous versus dentate transport segment sides; 3) the number of blood vessels and nerves in the regenerate versus the native bone behind the dentate and edentulous transport segments; and 4) the number of blood vessels and nerves in regenerate created by dentate versus edentulous BTDO.

Materials and Methods

Preliminary phase

This study utilized tissue specimens obtained from a previous study conducted by Malavia et al. (personal communication). During the initial study, five adult foxhounds, weighing approximately 70 pounds each, were subjected to a mandibular surgical procedure in which the anterior portion of the mandible was removed, creating a defect much larger than 60 to 70 mm, which is a critical size defect. A novel bone transport reconstruction device (BTRP-02, Craniotech ACR devices, LLC) (Figure 1) was used to regenerate the bone within the gap. Specifically, two transport segments of a similar size were created on each side of the mandible. One of the sides carried an endodontically treated tooth, and the contralateral side was edentulous. The fourth premolar on the left side was extracted one week prior to surgery. On the right side of the mandible, the fourth premolar was endodontically treated one week prior to surgery. After the latency phase, the device was activated at a rate of 1mm per day under light sedation through the distraction period. The distraction was continued until both transport segments met in the midline, creating a docking site. During the consolidation period, the dogs were injected three times with bone markers every two weeks with the last marker injected one week before sacrifice. The markers used were oxytetracycline (Tetradure 300) 25 mg/kg, IV (Merial Ltd) and calcein 10 mg/kg, IV (Sigma-Aldrich) with the latter injected as the first and last label. After approximately 40-44 days of consolidation, the animals were sacrificed using beuthanasia-D (1cc IC while under anesthesia) in accordance with the recommendations of the panel on Euthanasia of the American Veterinary Medical Association. All surgical procedures were described in detailed by Malavia et al., in part 1 of this study (Malavia et al., personal communication).

Specimens

The mandibles were resected en bloc, sectioned into left, right and anterior portions, and placed in 70% ethanol (ETOH). The samples were then dehydrated using different concentrations of ETOH (ascending from 70% to 100%) and embedded in methyl methacrylate. The specimens were sectioned with a Buehler wafering saw to create slices (120 microns thick). Between one and eighteen sections were made for each segment. After the processing of the specimens, four sections were selected from both the right and left side of the mandible: one native bone (NB), one posterior regenerate bone (PRB), one middle regenerate bone (MRB), and one anterior regenerate bone (ARB); one specimen was selected for the midline (Figure 2). The criteria used for the selection were: 1) NB including a section through the tooth, 2) PRB, with the first section showing definitive initial differentiation of regenerate bone at the edge of the native bone, 3) MRB, with one section located at the middle of the regenerate bone, and 4) ARB was the last portion of the regenerate bone (Figure 3). A single anterior midline specimen was selected based on the closest bone proximity of the two transport discs in the midline.

Fluorescence microscopy and MAR

The sections were analyzed with fluorescence microscopy to calculate the mineral apposition rate (MAR) by measuring the distances between the fluorescent bone markers, calcein and tetracycline. Images of the bone markers were captured using the Leica TCS SP511 confocal laser microscope, which emits a laser light at a specific wavelength (390 Nm to 470Nm) to reveal the markers. Each section was examined at two magnifications. At 2.5X magnification, images of the whole specimen were taken of one of the dogs (Figure 4). At 5.0X magnification, four images were taken of each specimen as specified in Figures 5,6 and 7 (buccal, lingual, superior, inferior zones)

Using the Microsoft image composite editor (ICE-1.4.4) program, the images were stitched together to create the image of the complete specimen.

The images taken at 5.0X magnification (buccal, lingual, superior and inferior zones) were analyzed with Bioquant software (Bioquant Image Analysis Corp., Nashville, TN). This software was used to quantify the MAR of the newly formed bone. Three regions were selected randomly from each zone for the MAR measurements. The criteria used for selecting the bands to measure, was based on the clearest demarcation between the fluorescent bone markers and the largest distance between them within the region of interest (Figure 8). The measurements for each slide were made twice with the minimal separation period of one day between each measurement.

Statistical comparisons were made between the native and regenerate bone on each side of the mandible (dentate vs. edentulous). A comparison was also made between the regenerate bone on the left and right sides (dentate vs. edentulous).

Histology and quantification of blood vessels and nerves

After the fluorescent images were captured, the sections were stained with Stevenel's blue and Van Giesen picro-Fuschin red for histologic examination and for counting the nerves and blood vessels. Cells and extra cellular structures stained blue while bone and other calcified tissues stained red (Figure 9). The stained sections were used to quantify the number of blood vessels and nerves present in the alveolar canal. Images of the slides were captured using an Olympus DP72 Microscope Digital Camera (Pennsylvania,USA) at 10X magnification, and the images were stitched together using the Microsoft image composite editor (ICE-1.4.4) to capture the image of the complete specimen. These images were analyzed with Bioquant software (Bioquant Image analysis Corp, Nashville, TN). The blood vessels and nerves were classified as follows: a) Large blood vessels, in which small blood vessels (vasa vasora) were found in the adventitia (Figure 10A); b) small blood vessels, that did not contain vasa vasora (Figure 10B); c) large nerves, in which blood vessels were visible in the perineurium (11A); and d) small/medium nerves where no blood vessels, were visible in the perineurium (Figure 11B). The quantification of the blood vessels and nerves was initially done three times in ten of the specimens before the final count to prove the repeatability of the measurements.

Statistical analysis

SPSS statistical software (version 17.0. Chicago:SPSS Inc) was used to analyze and compare the MAR, blood vessels and nerves between the native bone and the regenerate bone. One-way ANOVA was used to compare the MARs on the dentulous and edentulous sides, as well as for comparisons between the native bone and regenerate bone. Post hoc T-test comparisons using Sidak's adjustment were used to compare mineralization rate between the native and regenerate bone groups. A Kruskall-Wallis test was used to compare the blood vessel and nerve count between native and regenerate bone. A Mann-Whitney test was used for comparisons between the dentate and edentulous sides. For all analyses, the statistical significance was set at $\rho \leq 0.05$.

Results

Missing data

After the decalcification process, some of the specimens were damaged and could not be observed under the microscope. The numbers of sections analyzed for each region are given in the Tables.

Overview of the results

Differences in the cortical bone and neurovascular bundle were noted between the native bone and the regenerate bone. The cortical bone was thick and well defined in the native bone, and the cortex became thinner and less clear as the bone transitioned to regenerate. This thinning continued more medially towards the docking site in the symphysis area (Figure 3). The neurovascular bundle appeared compact and well defined in the native bone. In the regenerate it appeared more disorganized and dispersed within the medial and anterior segments. Continuity of the alveolar blood vessels and nerves from the native bone was noted even in the most anterior regenerate segments (Figure 12).

Mineral Apposition Rate (MAR)

The MAR was evaluated using images captured by confocal microscopy. In all, 207 sites were analyzed (NB=76, PRB=53, MRB=57, ARB=21). The native bone had a mean MAR of 3.7 ± 1.0, while the regenerate bone had MAR means of PRB 4.6 ± 1.6, MRB 4.4 ± 1.3, ARB 4.8 ± 1.3 (Table 1). There was a significant statistical difference in the MAR between the native bone and the regenerate bone (PRB, MRB, ARB) on either side of the mandible (edentulous or dentulous) (Table 1). In the overall comparison of regenerate bone, the mean MAR for the dentulous side was 4.5 ± 1.5 μm and for the contralateral side was 4.7 ± 1.4 μm. Significant differences in MAR between the different regions and segments of the regenerate bone were not observed (Table 2).

Blood vessels and nerves

Images were captured for the histologic analysis at 10X magnification. There were many more small nerves and blood vessels than large nerves and blood vessels in all the segments examined. No significant differences were found in the comparison of blood vessels (large or small) between the regenerate and native bone (Table 3). In the overall comparison of the regenerate bone (dentulous or edentulous), no significant differences were found in the number of large or small blood vessels (Table 4).

The nerves were also compared and no statistically significant differences were found in the numbers of large or small nerves between the native and regenerate bone (Table 5). In the comparison of the different regions of regenerate bone (dentulous and edentulous), no significant statistical differences were found in the number of nerves (Table 6).

Discussion

In this study bone transport distraction osteogenesis (BTDO) was used to fill a bone gap, in which a bone segment called the “transport disc” was transported along the defect. This study used an approach in which two transport discs were created, one on each side of a mandibular defect, and then they were moved until they meet in the midline at the symphisis. The purpose of the study was to compare histologically the blood vessels and nerves within the alveolar canals of the regenerate bone created by BTDO with one transport disc containing an endodontically treated tooth and the other a partially healed extraction socket. In addition, the MAR within the regenerate bone on either side was compared.

No differences were observed in numbers of blood vessels and nerves within the alveolar canals of the regenerates created behind edentulous and dentate transport segments, regardless of the position in the regenerate- posterior, medial or anterior in the mandible. No differences in MAR were noted between regenerates from edentulous er dentate transport segments. However the MAR was significantly different between the regenerate and the native bone. The higher MAR in regenerate bone suggests that the regenerate is still relatively immature bone in the early stages of mineralization, and is still actively remodeling. These differences were significant after six weeks of consolidation. Higher MAR numbers at the end of the consolidation period have been reported by others (Cope and Samchukov 2000; Cope and Samchukov 2001). Cope et al. conducted two independent studies with beagle dogs to measure the MAR at different periods of DO. In one of the studies, a defect was created, and the mineralization dynamics were measured by plain film radiography and digital subtraction radiography; in the other study, mineralization was measured with two different markers by injecting two the dogs at different periods. Both studies found that the MAR was higher as the weeks of consolidation progressed. The measurements were done from 0 to 8 weeks (Cope and Samchukov 2000; Cope and Samchukov 2001). One of the studies also found that the mineral apposition rate was higher in the zones that were closer to the native bone (Cope and Samchukov 2000). They attributed this situation to a richer vasculature closer to the native bone.

In BTDO studies, as well as in DO, the consolidation period plays an important role in the amount of mineralization seen in the new bone (Kontogiorgos, Elsalanty et al. 2011; Zapata, Halvachs et al. 2011; Zapata, Opperman et al. 2011; Nagashima, Rondon-Newby et al. 2012). Nagashima et al. used foxhound dogs and created a unilateral 34 mm defect that was reconstructed with BTDO. After 4 weeks of consolidation, the histologic and biomechanical characteristics of the regenerate were compared to the control bone. It was found that after a month, the regenerate bone was less mature, and the mineralization process was still ongoing (Nagashima, Rondon-Newby et al. 2012). Kontogiorgos et al. created a unilateral 3 to 4 cm defect in adult foxhounds that was reconstructed with BTDO. After the distraction period, they used micro-computed tomography to assess the morphometric and structural indices of the regenerate bone. They showed that the regenerated bone was still mineralizing at 18 weeks of consolidation (Kontogiorgos, Elsalanty et al. 2011). In a similar study using Micro-CT analysis and histology of the regenerate bone, Zapata et al. concluded that BTDO was able to successfully generate new cortical bone similar to the original bone after 18 weeks of consolidation (Zapata, Halvachs et al. 2011). In the same study a second group of dogs were sacrificed after 12 weeks of consolidation. The biomechanical characteristics of the regenerate and native bone were analyzed. It was concluded that the differences between native and regenerate bone after the consolidation period were due to different levels of mineralization (Zapata, Opperman et al. 2011). These studies showed that the higher the mineralization of the regenerate bone is, the longer the consolidation period. This finding is in accordance with the results of this study, which showed that after 6 weeks of consolidation, the regenerate bone was still mineralizing.

Histological analysis of the blood vessels and nerves within the alveolar canal showed no significant difference between the regenerate and native bone, or between the dentate and edentulous regenerates. No significant differences were found in the number of blood vessels when native and regenerate bones were compared. One of the patterns observed was that there were more small blood vessels than large blood vessels, both in the native bone behind the regenerate and throughout the regenerate. However, there was always the presence of at least one large artery within the alveolar canal, both in the regenerate from dentate or edentulous transport segments. These results suggest that the inferior alveolar artery was successfully transported and regenerated along the new regenerate bone. Although similar results have been reported in mandibular DO (Costantino Pd and et al. 1990; Karp, Thorne et al. 1990) ,it is important to emphasize that with BTDO nerves and blood vessels anterior to the transport disc are severed. This could increase the risk of the neurovascular bundle not to regenerate which can compromise the outcome of the surgical procedure. Costantino et al. demonstrated that with DO after the complete resection of the neurovascular bundle on a segment of the mandible, the inferior alveolar artery was regenerated along the new bone (Costantino Pd and et al. 1990). One explanation for the constant number of smaller blood vessels can be that the specimens had a consolidation period of approximately 6 weeks in which the proliferation of blood vessels decreases (Rowe, Mehrara et al. 1999; Choi, Ahn et al. 2000). Choi et al. showed in a rat model that at 7 and 14 days of distraction, there was a proliferation of blood vessels. This vascular proliferation occurred actively during the latency and distraction periods and then gradually decreased over time (Choi, Ahn et al. 2000). Rowe et al. also suggested that blood vessel proliferation was observed in the early stages of distraction and then decreased during later stages. It was observed that the blood vessels were more mature and bigger at these later stages. They mature during the consolidation period and simulate the vessels from the native bone (Rowe, Mehrara et al. 1999).

The results of the present study indicate that the inferior alveolar nerve was transported along the regenerate bone in addition to the blood vessels. As mentioned before it is important to highlight the fact that the neurovascular bundle was completely cut anterior to the transport disc. Due to the gap in information regarding the study of nerve formation in BTDO only one study was reviewed with similar conditions. Isomura et al. created a 10mm defect on one side of the mandible, leaving the contralateral side as control (Isomura, Shogen et al. 2013). Using bifocal distraction osteogenesis, the transport disc was moved along the defect at a rate of 1mm/day, regenerating a 10mm mandibular defect including the inferior alveolar nerve (IAN). In their study they suggested that a 30 day period was needed for the regenerated nerve to sprout into the docking site area of the transport disc. On the other hand DO studies have shown that the nerve can be transported along the regenerate bone (Block, Daire et al. 1993). Block et al. found that the inferior alveolar nerve spanned the regenerate bone without a continuity defect. Karp et al. tried to preserve the neurovascular bundle in their experiment, and they also found regeneration of the inferior alveolar nerve. Furthermore, they reported the absence of myelinated fibers on the regenerated side, which indicates the possible impairment of nerve function (Karp, Thorne et al. 1990). In contrast, myelinated fibers were observed in the regenerated IAN of our specimens, which indicates that the nerve was successfully transported. Although function was not tested in our experiment it can be hypothesized that the presence of myelinated fibers indicates at least some preserved function of the nerve. Conversely, Costantino et al. intentionally cut the neurovascular bundle by performing an osteotomy to separate the transport disc from the native bone and found that even though the anterior segment of the mandible was still present the inferior alveolar nerve had not regenerated after 8 weeks of consolidation (Costantino Pd and et al. 1990). Therefore DO may be associated with abnormalities in the inferior alveolar canal that can affect the function of the patient after a rehabilitation. Makarov et al. suggested that with DO, the size of the defect will affect the degree of regeneration of the nerve (Makarov, Harper et al. 1998). Although in this study a thorough examination of the nerve was not performed, the fact that the nerve regenerated along the whole anterior segment of the mandible could be attributed to the stretching of the nerve with the transport disc and the regeneration of the nerve in the inferior alveolar canal of the transport disc as was mentioned by Isomura et al. in his study.

The absence or presence of a tooth in the transport segment did not affect the numbers of blood vessels, nerves or mineral apposition rate in the regenerate bone. This finding is important because in actual reconstruction surgery, teeth that can be transported will provide the restorative dentist with more options in the rehabilitation process. Teeth that can be saved in extensive surgical resections can be used for retention and support of overdentures and removable dental prosthesis (RDP). Overdentures should be considered in the event of loss of alveolar bone support and with patients with a poor prognosis for complete dentures. RDP should also be considered as an option in which the remaining teeth can be used as abutments. In general when dealing with mandibular defects, everything that can be done to increase the potential usefulness of the remaining teeth should be done. One of the primary goals of treatment is the restoration of acceptable occlusal function. The success of the restoration is related to the location and extent of the mandibular resection and the presence or absence of natural teeth (Desjardins 1979). In this study, the tooth was transported on the transport disc up to the anterior segment of the mandible without any problems. The tooth didn’t suffer any lesion or problem that could jeopardize its future rehabilitation improving the prognosis of the treatment for the patient.

CHAPTER III

CONCLUSIONS

The purpose of this research was to quantify the number of blood vessels and nerves and mineral apposition rate(MAR) in native bone and compare it to the regenerate bone produced by bone transport distraction osteogenesis (BTDO).

The histologic analysis showed no significant differences in the number of blood vessels (large or small) and nerves (large or small) between the native and regenerate bone. Confocal microscopy and Bioquant analysis showed significant differences ($P \leq 0.05$) in the MAR between the native bone and the regenerate bone but no significant differences were observed between the dentate and edentulous regenerates.

In conclusion, this study suggests that BTDO be considered as a treatment option to reconstruct large mandibular defects where the nerves and blood vessels have been removed in the anterior part of the transport disc. We showed that the blood vessels and nerves are regenerated on the new bone. This presence of blood vessels plays an important role in the formation of new bone, as it is associated with a healthy blood supply.

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APPENDIX A

FIGURES

Figure 1. Mandibular bone transport reconstruction plate (BTRP) device. This device is a new intraoral device with a modified reconstruction plate working as a transport track, along which the transport bone disc travels towards the docking site. It allows two forms of distraction: transport can occur from one side of the mandible to a docking site on the other side, or two transport units on either side of the mandible can be transported towards the midline until they meet at the symphisis (Shown here).

Figure 2. Schematic drawing indicating the place were the mandible was resected into the different sections that were analyzed. **NB**, native bone. **PRB**, Posterior regenerate bone. **MRB**, Middle regenerate bone. **ARB**, Anterior regenerate bone.

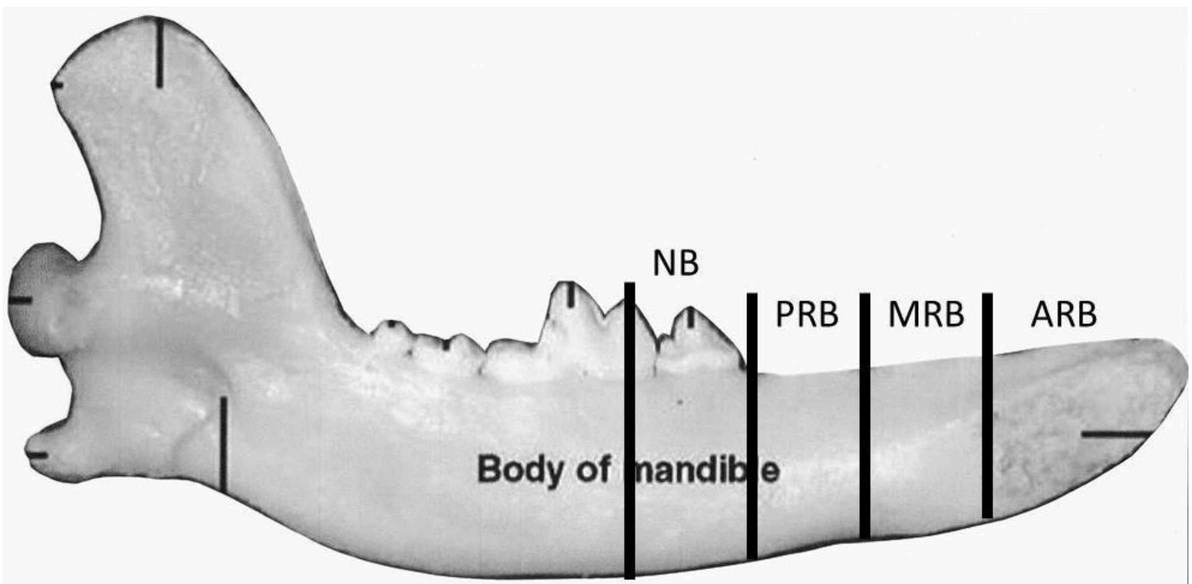


Figure 3. Composite of stained images photographed and calibrated with a milimetric ruler. Criteria used to select specimen of each section. A,NB including a section through the tooth where the cortical bone is thick and well defined. The cortex became thinner and less clear as the bone transitioned to the regenerate (B).B,PRB the first section showing definitive initial differentiation of regenerate bone. C,MRB section located at the middle of the regenerate were the cortical bone is less dense. D,ARB was the last section of the regenerate bone closest to the transport disc.

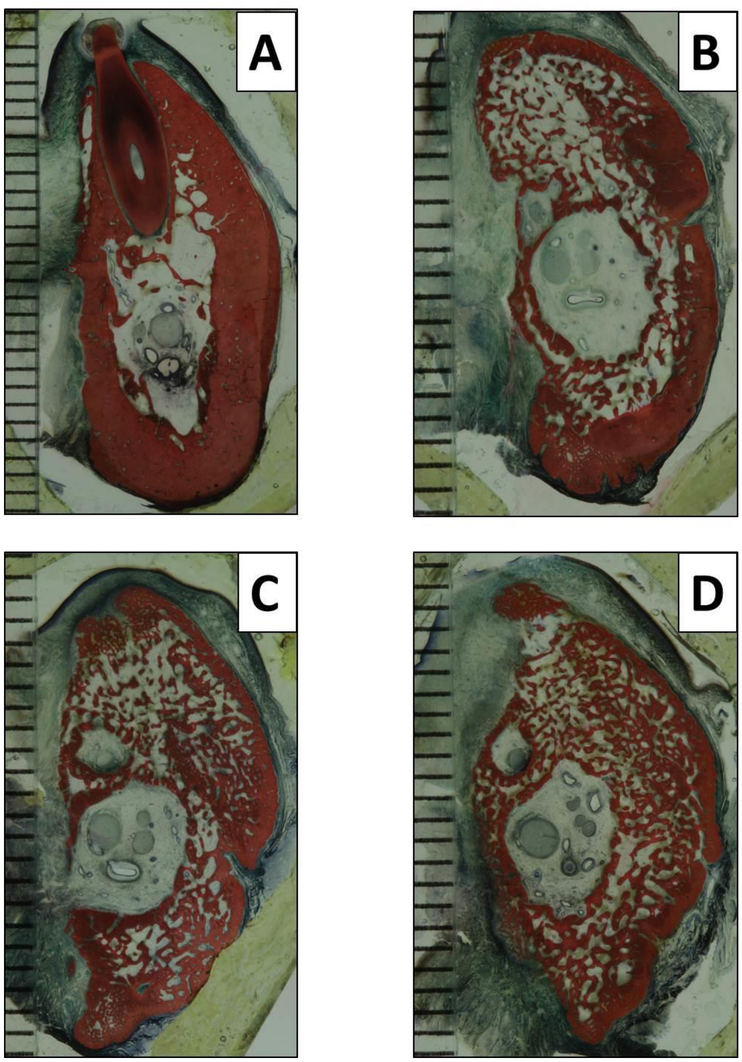


Figure 4. Fluorescence microscopy images taken with the confocal microscope at 2.5X magnification. A, NB. B,PRB. C, MRB. D,ARB.

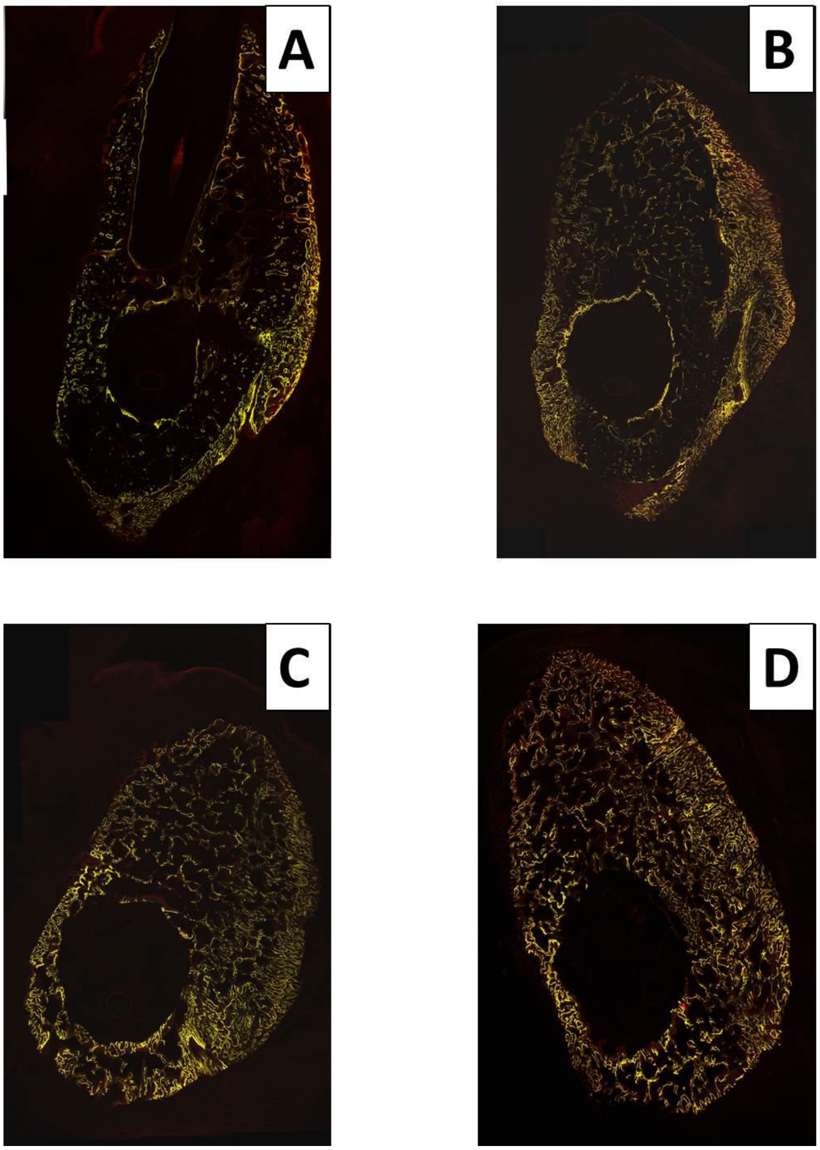


Figure 5. Fluorescent image of NB shown in figure 4 at 5.0X magnification. Four regions were selected for analysis of MAR. Red circles indicate buccal, lingual, superior and inferior sections that were utilized to quantify the rate of mineral apposition.

Figure 6. High power fluorescence image demonstrating how MAR was measured with the Bioquant software. Inside the red circle is a representation of how the different labels were outlined on the bone and how the software created perpendicular lines (green) to measure the MAR.

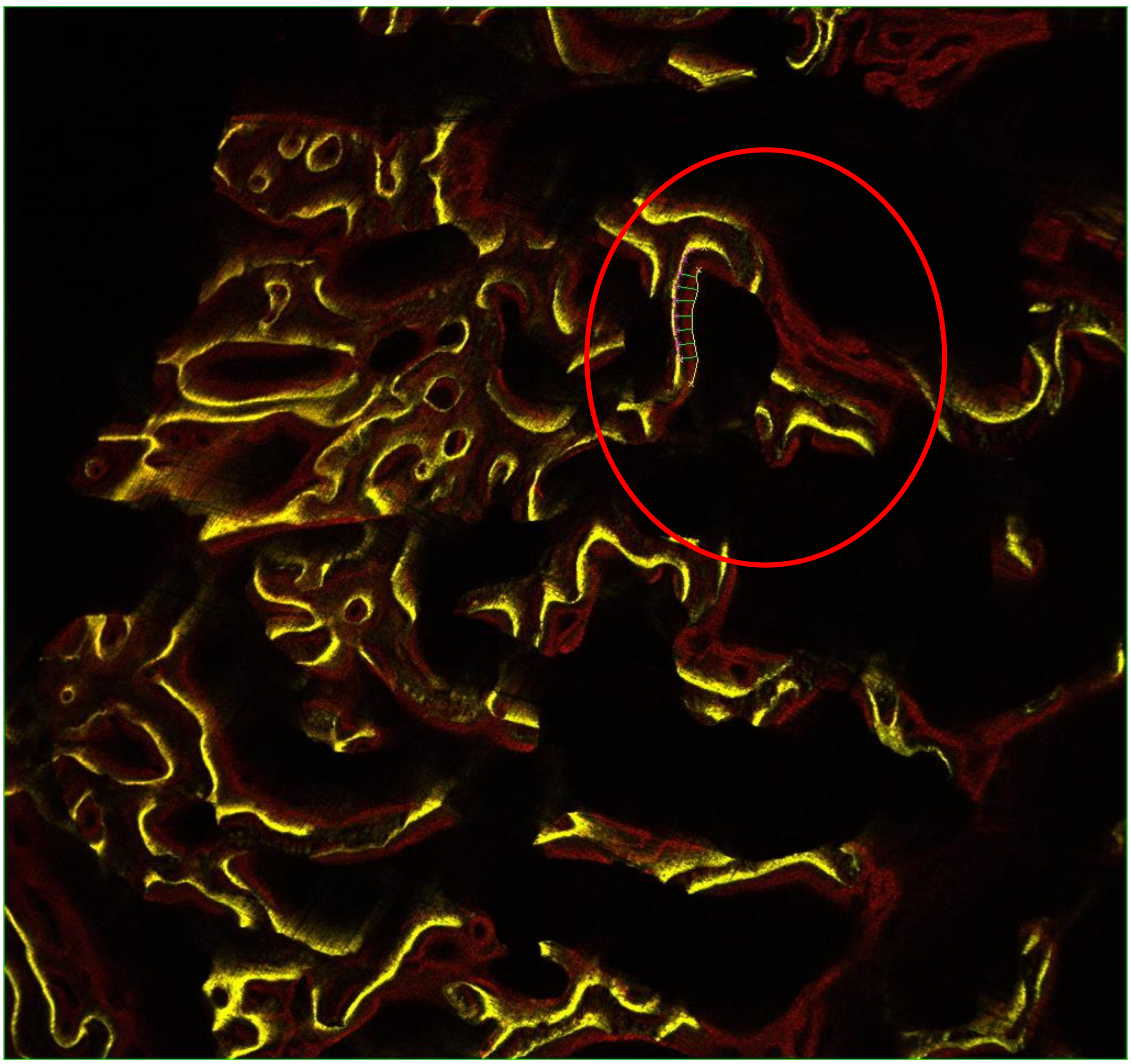


Figure 7. Stevenel's blue and Van Giesen picro-fuschin red stained section of native bone for histologic examination and for counting blood vessels and nerves. Cells and extracellular structures were stained blue while bone and other calcified tissues stained red.

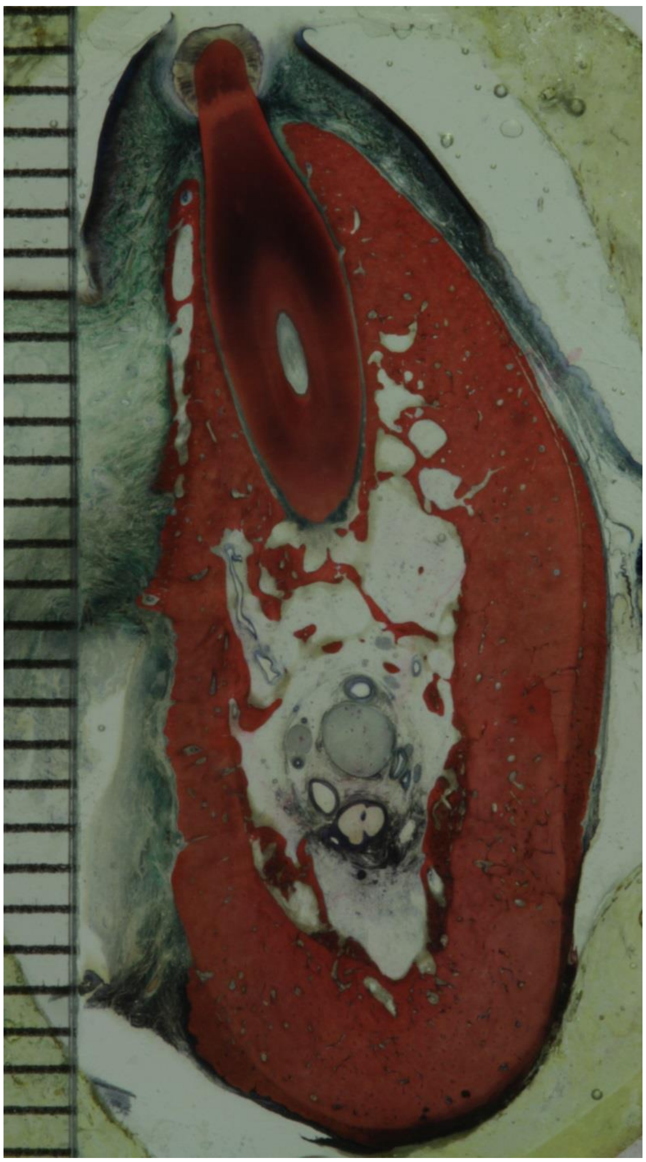


Figure 8. A,Low power (5X magnification) histological image identifying blood vessels in the alveolar canal. B, Magnified area (10X) in box B. Large blood vessel with associated small blood vessel (vaso vasora) found in the adventia marked with the black arrow. C, Magnified area (10X) in box C. Small blood vessel (black arrow) that did not contain vaso vasora in the adventia.

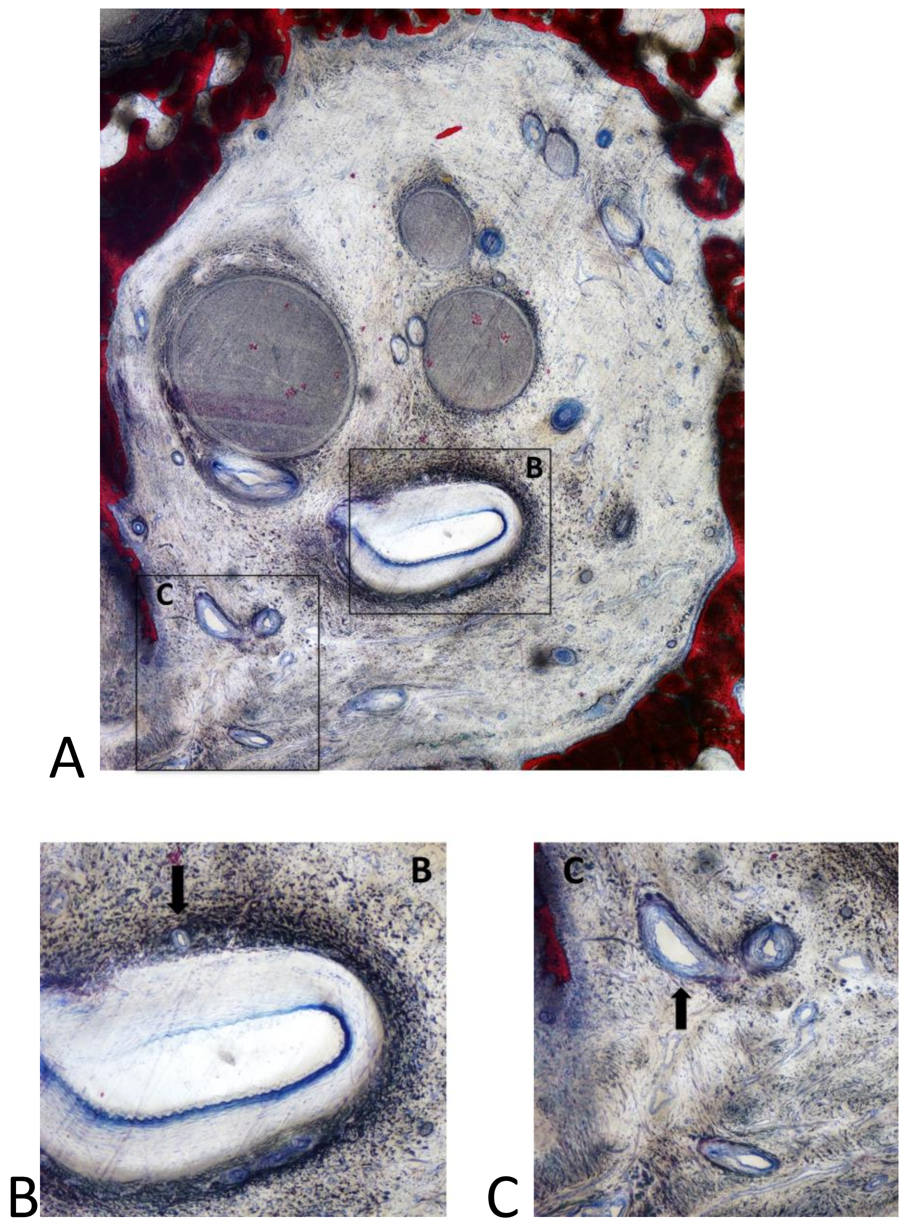


Figure 9. A,Low power (5X magnification) histological image identifying nerves in the alveolar canal. B, Magnified area (10X) in box B. Large nerve with associated small blood vessel (black arrow) visible in the perineurium. C, Magnified area (10X) in box C. Small/medium nerve (black arrow) were no blood vessels are visible in the perineuruim.

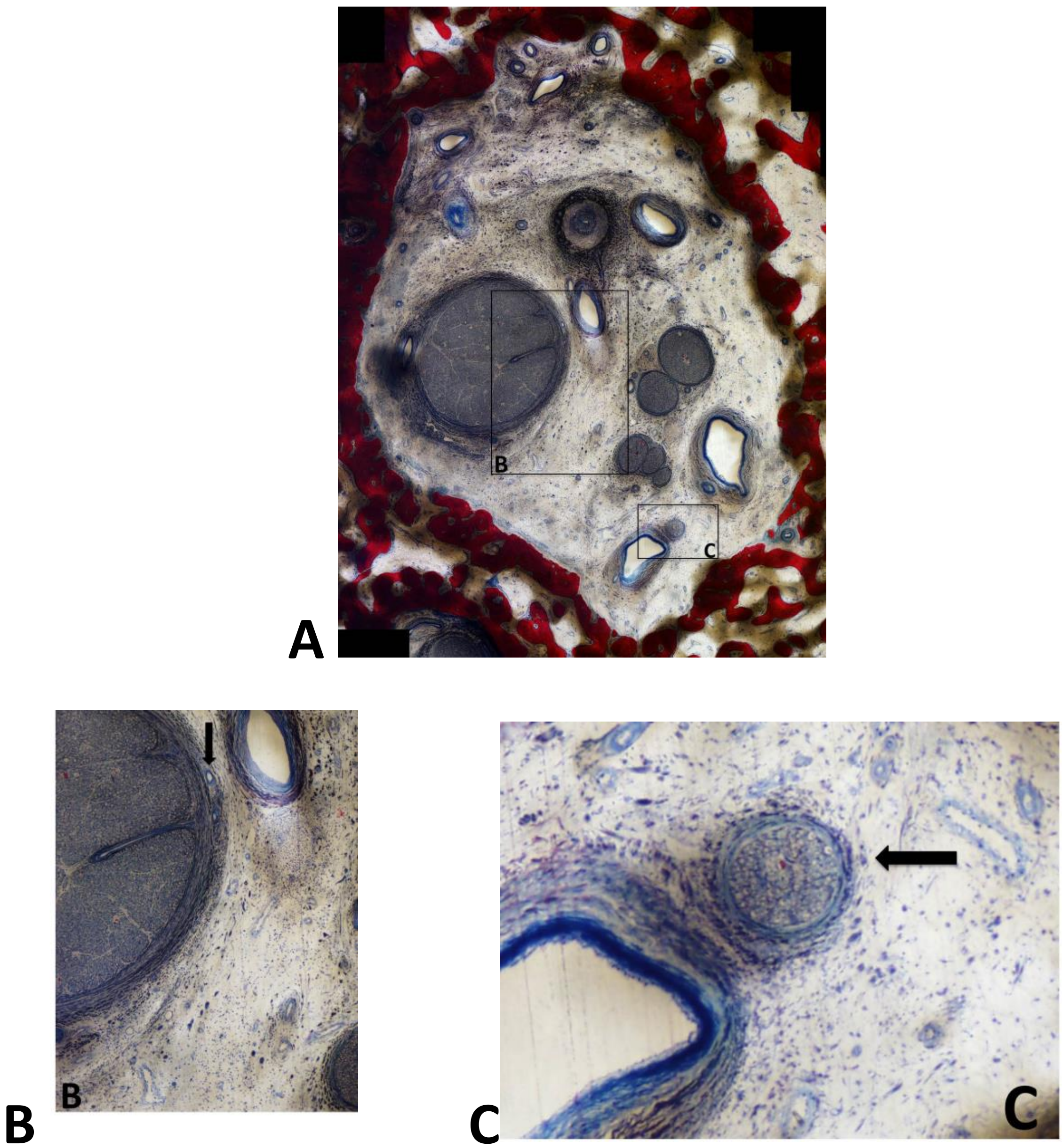
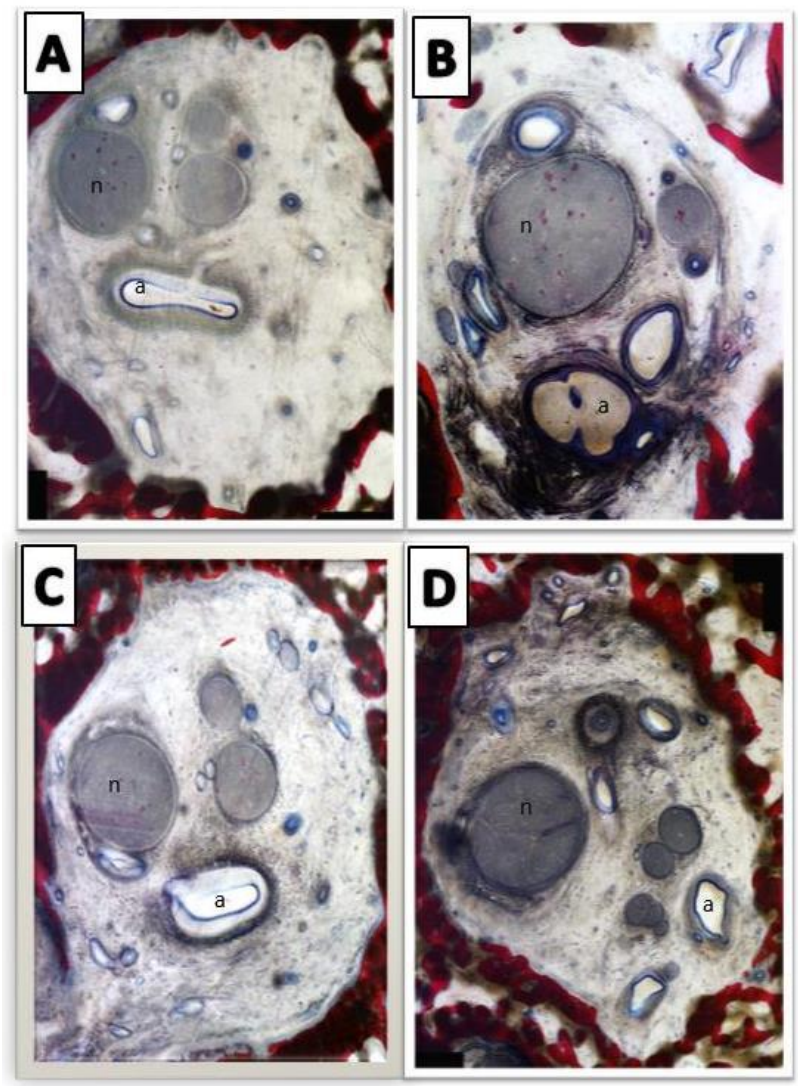


Figure 10. 1.25X magnification images of the neurovascular bundle from native bone (A) through the regenerate bone (B,C,D). A, Native bone were the neurovascular bundle is compact and well defined. The neurovascular bundle appears more disorganized and dispersed in sections more anterior within the PRB (B), MRB (C), and ARB (D) segments of the regenerate bone. Continuity of the alveolar blood vessels (a) and nerves (n) from the native bone (A) was noted even in the most anterior regenerate segments (D).



APPENDIX B

TABLES

Table 1. Descriptive statistics comparing the mineral apposition rate between the native and regenerate bone

	MAR (µm)			
Descriptive Statistics	**Native Bone (n=76)**	**Posterior Regenerated Bone (n=53)**	**Middle Regenerated Bone (n=57)**	**Anterior Regenerated Bone (n=21)**
Median	3.6	4.5	4.0	4.3
Mean	3.7*	4.6*	4.4*	4.8*
SD	1.0	1.6	1.3	1.3
Minimum	1.7	1.8	2.6	2.8
Maximum	5.8	9.8	8.4	7.0

*P<0.05

Table 2. Descriptive statistics comparing the mineral apposition rate between dentate and edentulous regenerate bone.

	MAR (µm)	
Descriptive Statistics	**Edentulous (n=73)**	**Dentulous (n=58)**
Median	4.1	4.6
Mean	4.5	4.7
SD	1.5	1.4
Minimum	1.8	2.4
Maximum	9.8	8.4

Table 3. Descriptive statistics comparing the number of blood vessels between the native and regenerate bones.

Descriptive Statistics	Native Bone (n=6)		Posterior Regenerated Bone (n=7)		Middle Regenerated Bone (n=8)		Anterior Regenerated Bone (n=7)	
	Blood Vessels Count		Blood Vessels Count		Blood Vessels Count		Blood Vessels Count	
	Small	Large	Small	Large	Small	Large	Small	Large
Median	26	2	35	1	40	1	31	1
Mean	35	2	31	1	37	1	35	2
SD	19	1	8	1	11	0	12	1
Minimum	20	1	20	1	14	1	17	1
Maximum	63	3	38	3	51	2	52	3

Table 4. Descriptive statistics comparing the number of blood vessels between the dentate and edentulous regenerate bone.

Descriptive Statistics	Edentulous (n=9)		Dentulous (n=13)	
	Blood Vessels Count		Blood Vessels Count	
	Small	Large	Small	Large
Median	31	1	38	1
Mean	32	1	36	1
SD	11	1	10	1
Minimum	17	1	14	1
Maximum	51	3	52	3

Table 5. Descriptive statistics comparing the number of nerves between the native

Descriptive Statistics	Native Bone (n=6)		Posterior Regenerated Bone (n=7)		Middle Regenerated Bone (n=8)		Anterior Regenerated Bone (n=7)	
	Nerves Count		Nerves Count		Nerves Count		Nerves Count	
	Small	Large	Small	Large	Small	Large	Small	Large
Median	15	2	8	2	9	2	11	2
Mean	14	2	8	3	9	2	10	3
SD	5	1	5	2	3	1	3	3
Minimum	8	1	1	1	4	1	6	1
Maximum	21	3	14	6	12	4	13	8

*P<0.05

Table 6. Descriptive statistics comparing the number of nerves between the dentate and edentulous regenerate bone.

Descriptive Statistics	Edentulous (n=9)		Dentulous (n=13)	
	Nerves Count		Nerves Count	
	Small	Large	Small	Large
Median	10	3	8	2
Mean	9	3	9	3
SD	4	2	3	2
Minimum	1	1	6	1
Maximum	13	6	14	8